Qualification and Quantification of N-nitrosodimethylamine using Ultrahigh Performance Liquid Chromatography with Tandem Mass Spectrometry

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Nitrosamine impurities in human medicines have raised concern among global health regulators because many of these compounds are known to be strong mutagens and carcinogens. N-nitrosodimethylamine (NDMA) is a nitrosamine that has been detected in several products, raising safety and quality concerns. The purpose of this study was to develop and validate a simple, reliable, and highly sensitive liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for detecting and quantifying trace levels of NDMA in Ibrutinib, a medicine used to treat certain cancers. The chromatographic separation was carried out on an XSelect CSH Phenyl-Hexyl column (150 × 4.6 mm, 3.5 µm) using gradient elution with 0.12% formic acid in water as mobile phase A and methanol as mobile phase B. Detection was performed using atmospheric pressure chemical ionisation (APCI) in positive mode, operating under multiple reaction monitoring (MRM) conditions at m/z 75 and 58 for NDMA. The method showed very good sensitivity with a limit of detection (LOD) of 0.005 ppm and a limit of quantitation (LOQ) of 0.01 ppm. The method was validated in accordance with the International Council for Harmonization (ICH) guidelines and demonstrated accuracy, precision, linearity, specificity, and robustness across the tested range. These validation results confirm that the method is suitable for the routine determination of NDMA impurity in Ibrutinib. In conclusion, the developed LC-MS/MS method provides a reliable and efficient tool for monitoring NDMA contamination at very low levels, supporting regulatory compliance and ensuring the safety and quality of pharmaceutical products. The method can also be adapted for the analysis of NDMA in other drug substances and formulations where similar contamination risks may exist.

Keywords: N-nitrosodimethylamine, NDMA, genotoxic impurity, Ibrutinib, UPLC-MS/MS

Introduction

Since 2018, leading regulatory authorities (EMEA, FDA) have required that, for some angiotensin II receptor blockers (belonging to a class of medicines called sartans) and later some other medicines (histamine antagonists, metformin, nitroglycerin), the regulation and control of nitrosamine impurity levels are mandatory quality and safety characteristics. Nitrosamine impurities are compounds containing a nitroso group at a dialkyl-substituted amine group. The primary danger from nitrosamine impurities is that they are compounds with potential genotoxicity, meaning reliable information on genotoxicity has been obtained in animal studies, but limited or insufficient information on human toxicity. Nitrosamine impurities are included in the “cohort of concern” compounds, which means that the general approach for determining acceptable concentrations based on toxicological value threshold is not applicable in this case. The crisis emerged following significant recalls of valsartan tablets due to nitrosamine impurities, followed by the withdrawal of ranitidine in several countries, widespread metformin recalls from the market, and a growing list of other medicines found to contain nitrosamine impurities [1-5].

The Threshold of Toxicological Concern (TTC), established at 1.5 µg/day for pharmaceuticals, defines an acceptable patient daily intake for any unstudied chemical posing a negligible risk of carcinogenicity or other toxic effects. A group of highly potent mutagenic carcinogens, defined solely by the presence of structural alerts, is referred to as the “cohort of concern” (CoC); compounds such as aflatoxin-like-, N-nitroso-, and alkyl-azoxy derivatives are considered to pose a significant carcinogenic risk at intake levels below the TTC. Kroes *et al.* (2004) [6] derived values for both TTC and CoC for food components, based on a non-transparent dataset that was never made publicly available. Using a reconstructed dataset of all carcinogens from relevant publications, it has become clear that exceptions exist across all three CoC structural classes. Within the reconstructed dataset, N-Nitrosamines account for 62% of the N-nitroso class. An investigation of a contemporary dataset indicates that ~20% of these compounds are negative in rodent

carcinogenicity bioassays, and that less than 50% of all N-nitrosamines are estimated to fall into the highest risk category. Therefore, it is recommended that CoC nitrosamines be identified based on compound-specific data rather than structural alerts. This approach allows distinguishing CoC from non-CoC N-nitrosamines in the context of mutagenic impurities as described in ICH M7 (R1) [6-8].

Ibrutinib (Figure 1), an orally administered drug sold under the brand name “Imbruvica”, is a first-in-class irreversible covalent inhibitor of Bruton’s tyrosine kinase (BTK), used primarily for treating B-cell malignancies. It is approved for the management of chronic lymphocytic leukemia and Waldenström’s Macroglobulinemia. Chemically, ibrutinib is identified as 1-{(3R)-3-[4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo [3,4-d]pyrimidin-1-yl] piperidin-1-yl}prop-2-en-1-on, and its molecular formula is $C_{25}H_{24}N_6O_2$. This small molecule binds irreversibly to the BTK protein, inhibiting B-cell proliferation and survival. By blocking the B-cell receptor pathway, which is often hyperactive in B-cell cancers, ibrutinib is effective against various conditions, including mantle cell lymphoma, chronic lymphocytic leukemia, and Waldenström’s macroglobulinemia. However, ibrutinib also binds to off-target proteins, such as C-terminal Src kinases. Its interaction with these kinases inhibits their ability to promote cell differentiation and growth, leading to various side effects. These adverse effects can include left atrial enlargement and atrial fibrillation during the treatment of chronic lymphocytic leukemia [9-11].

N-nitrosodimethylamine (NDMA) (Figure 1) is a colorless to light yellow oily liquid in nature, having a molecular formula of $C_2H_6N_2O$ and a molecular weight of 74.08. The impurity is soluble in chloroform, slightly soluble in ethyl acetate, and methanol. NDMA is a highly toxic, semi-volatile organic compound and a suspected human carcinogen. It has been shown to induce liver tumors in rats after chronic exposure to low doses. NDMA is listed on the Drinking Water Contaminant Candidate List 3 (CCL 3) by the United States Environmental Protection Agency (EPA) and is recognized as both an environmental and food contaminant. The acceptable interim concentrations of major nitrosamine impurities for active pharmaceutical ingredients are provided on the FDA website, based on animal toxicity studies. The safety levels for NDMA are established at 96 ng/day [12-13].

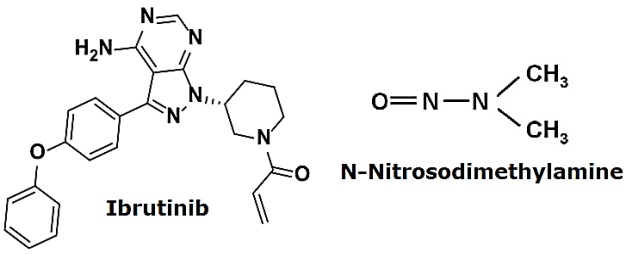

Fig.1. Structure of Ibrutinib and NDMA.

A number of highly sensitive analytical techniques for Genotoxic Impurities (GTIs) have already been developed. The majority of these analytical techniques use a mass spectrometry detector, which, in combination with a gas or liquid chromatography system, can identify traces of impurities [14-16].

So far, analytical methods for quantifying ibrutinib in bulk, formulations, and various biological matrices have been extensively described in the literature, primarily using LC-MS and other methods [19-20]. Given the growing regulatory focus on the determination of genotoxic impurities in pharmaceuticals, it is important to develop advanced, reliable analytical methods. Since no analytical method has so far been reported for the quantification of the NDMA impurity in the ibrutinib active pharmaceutical ingredient at the target analyte level of 0.03 ppm. Thus, the present study aims to develop a highly specific, sensitive, accurate, cost-effective, and direct LC/MS method for this target.

Experimental part

Materials

The NDMA standard was sourced from Sigma Aldrich. LC-MS-grade formic acid and methanol were purchased from Merck Millipore, and HPLC-grade water was generated using a Milli-Q water purification system (Merck). Samples of the ibrutinib substance were supported by Auxiliary Biosciences India.

Methods

An AB SCIEX QTRAP 4500 triple quadrupole mass spectrometer, equipped with an atmospheric pressure chemical ionization (APCI) probe, was used to develop and validate the LC-MS/MS method. Data acquisition and processing were handled using Analyst software.

LC-MS chromatographic conditions

Chromatographic separation was performed using an XSelect CSH Phenyl-Hexyl column (150 × 4.6 mm, 3.5 µm particle size). A flow rate of 0.8 mL/min was used for the gradient elution, with the mobile phase consisting of 0.12% formic acid in water (mobile phase A) and 0.12% formic acid in methanol (mobile phase B). The gradient program was applied. The column oven was maintained at 30°C, and the injection volume was 50 µL. Degassed methanol served as the diluent.

The mass spectrometer was operated in APCI positive mode, employing multiple reaction monitoring (MRM) for analyte detection. The transition ion pairs for NDMA were selected as *m/z* 75.1 > 58.1 for quantification and *m/z* 75.1 > 43.1 for qualification (Identification). The optimized MS/MS detector settings were a declustering potential (DP) of 42 V, an entrance potential (EP) of 15 V, and an ion source temperature of 350°C. A summary of the final liquid chromatographic and mass spectrometric conditions is provided. (Table S1, *Supplementary Materials*) (Figure 2).

Impurity standard and test sample solutions preparations

The primary stock solution for the impurity standard

was prepared in methanol at a concentration of 0.1 ppm. A working impurity standard solution was then prepared by serial dilutions with the diluent to achieve a concentration of 0.03 ppm, corresponding to the target analyte level relative to the sample concentration. Test samples of ibrutinib were prepared by dissolving the substance at approximately 100 mg mL^{-1} in the diluent, sonicating for about 5 minutes, and filtering the solution through a 0.45 µ polytetrafluoroethylene (PTFE) syringe filter before instrumental analysis.

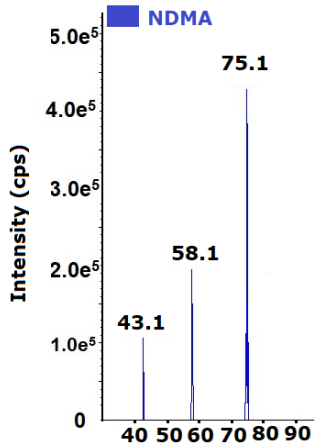

Fig. 2. NDMA mass fragmentation.

Results and discussion

Optimization of chromatographic conditions

The main goal of the current study was to quantify mutagenic impurity in the ibrutinib drug substance using an LC-MS method. Various diluents, including water, methanol, and acetonitrile, were assessed. Both NDMA and ibrutinib showed very poor solubility in water. They were moderately soluble in acetonitrile, but this resulted in a low response and split peaks for the analyte. Methanol provided superior solubility for both compounds and produced appropriate peak shapes in the chromatograms; consequently, it was chosen for sample preparation.

Different volatile buffers suitable for LC-MS analysis, such as ammonium acetate, formic acid, and ammonium formate, were tested as mobile phase A. Acetonitrile and methanol were evaluated as mobile phase B in a gradient elution mode. Several trials were conducted using these mobile phases and C18, C8, and phenyl columns. The combination of a formic acid buffer system and methanol provided good sensitivity and effective separation of the NDMA impurity from ibrutinib. Further trials utilizing various columns (Phenyl, Phenyl-Hexyl, C8, and C18) and different gradient programs were performed. Ultimately, the Phenyl-Hexyl column yielded the best peak shape for the impurity, enhancing retention, improving peak shape, and better recovery for low-molecular-weight compounds at trace-level concentrations.

The optimized conditions involve a gradient elution mode with a mobile phase system comprising 0.12% formic acid in water (mobile phase A) and methanol (mobile phase B). Separation was achieved using an XSelect CSH Phenyl-Hexyl column (150×4.6 mm, 3.5 µm particle size) with a total run time of 10 minutes. The NDMA impurity eluted at 2 minutes, and ibrutinib eluted at 5 minutes. To safeguard the mass spectrometer from the high concentration of ibrutinib, a diversion valve program was implemented to route the flow to waste after the ibrutinib peak eluted. (Table S1 *Supplementary Materials*) (Figure 2).

Optimization of mass spectrometric parameters

Selecting an appropriate detection method is a crucial aspect of pharmaceutical analysis. A triple quadrupole LC-MS/MS system, utilizing an APCI detector in MRM mode, provides significantly improved sensitivity and specificity for both screening and quantifying NDMA at sub-micromolar levels. This advanced system effectively addresses the limitations associated with less sensitive or specific analytical techniques. For NDMA analysis, specific mass conditions were selected based on characteristic transition ion pairs: m/z 75.1 > 58.1 was used for quantification, and m/z 75.1 > 43.1 for qualification. The mass spectrometry (MS/MS) detector parameters were set as follows: a declustering potential (DP) of 42, an entrance potential (EP) of 15, a collision energy (CE) of 24, and an MS temperature of 350°C. APCI detection provides superior sensitivity and a more stable response for NDMA, particularly at low molecular weights, compared to ESI detection. This integrated approach enables reliable trace-level quantification of NDMA, with improved performance. (Table S1 *Supplementary Materials*) (Figure 2).

Analytical method validation study

The developed analytical method was validated in accordance with the ICH, FDA, and USP guidelines [21-23]. The validation covered several key parameters, including system suitability, limit of detection (LOD), limit of quantitation (LOQ), specificity, linearity, accuracy, precision, and robustness. The results of this validation process are summarized below.

Specificity and system suitability

An analytical validation process was conducted to confirm the identity and retention time (RT) of the target analyte and to ensure reproducible system performance. This process included a system suitability test and an evaluation of specificity, followed by a system stability check.

For the specificity test, a blank sample, a spiked sample, a standard solution, and an individual standard prepared in the diluent were injected. Standards were prepared at a concentration of 0.03 ppm. The results showed that the relative standard deviation (%RSD) of the standard peak area was within acceptable limits, and no interfering peaks were observed at the analyte's RT. The analyte peak in the spiked sample, standard, and individual standard all eluted at the same retention time. These findings collectively demonstrate that the method is specific. (Table 1) (Figure 3).

Table 1. Summary of method validation results.

Validation parameters	Typical acceptance criteria	Results
System suitability	RSD (%) for peak area response (n = 6) should be ≤ 15.0.	2.6
Specificity	Retention time of the analyte in all the solutions.	2.0
	Interference from blank	No interference
LOD	Concentration of LOD in ppm	0.005
	s/n value should be ≥3	12
LOQ	Concentration of LOQ in ppm	0.01
	s/n value should be ≥10	28
LOQ precision	RSD (%) for six replicate injections of LOQ solution should be ≤ 15.0%	3.8
Linearity	Range (ppm)	0.01 to 0.06
	The correlation coefficient (r) should not be less than 0.99	0.998
	The square of the correlation coefficient (r^2) should not be less than 0.99	0.997

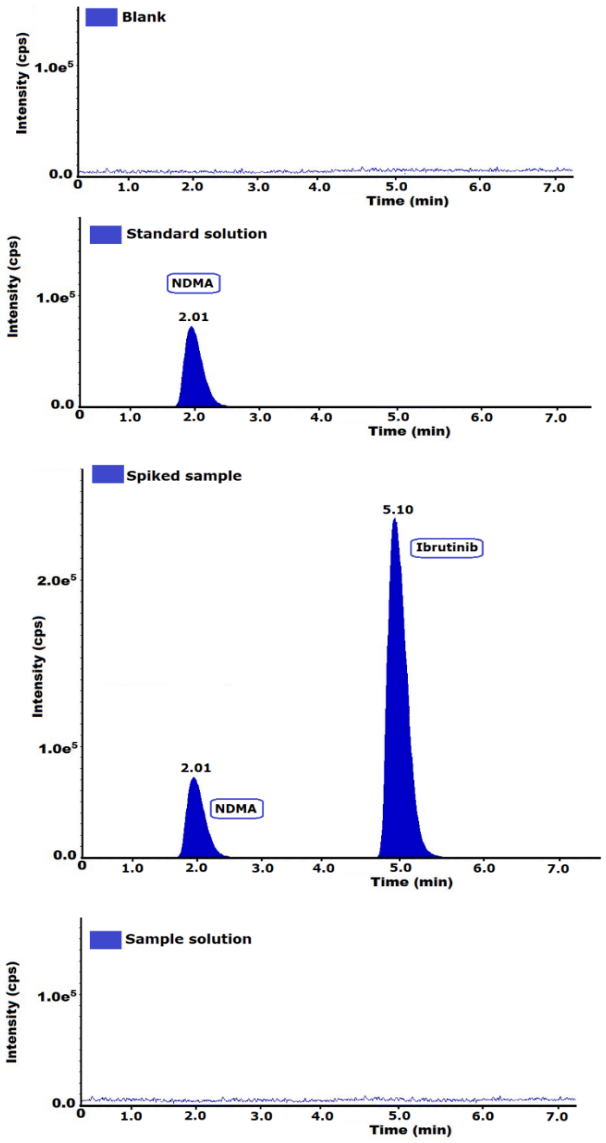

Fig. 3. MS/MS chromatogram of blank, standard, spike, and sample.

LOD, LOQ and LOQ precision

The established method's limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting diluted NDMA standard solutions at known concentrations in triplicate. The final LOD and LOQ values were established as 0.01 ppm and 0.05 ppm, respectively.

To confirm these limits, the signal-to-noise (S/N) ratio was verified. The LOD solutions demonstrated an S/N ratio of at least 3, while the LOQ solutions had an S/N ratio of at least 10, both meeting the acceptable criteria. Additionally, LOQ precision was assessed by performing six replicate injections of the LOQ solution. The calculated area %RSD for these replicate injections was 3.8% for the NDMA peak. (Table 1).

Linearity and Range

Linearity was established over a range of NDMA concentrations relative to the sample concentration, spanning from LOQ up to 200%. Five different known concentrations LOQ, 50%, 100%, 150%, and 200%, were prepared and injected in duplicate. A linearity graph was subsequently plotted, correlating the peak responses with the corresponding concentrations. The resulting correlation coefficient (r) was 0.998, and the square of the correlation coefficient (r^2) was 0.997 for NDMA. These results confirm that the method is linear (Table 1).

Method precision

A study was conducted to establish method precision (MP) using two sets of samples. Six unspiked samples were prepared, along with six additional samples spiked with 0.03 ppm NDMA at the specification level. All solutions were analyzed by a single injection per preparation. This was done to assess the reproducibility of the analyte content in the spiked samples and to determine the %RSD for NDMA.

The unspiked samples showed no detectable NDMA. The results for the spiked solutions demonstrated repeatability, with an obtained content %RSD of 3.2% for the NDMA peak. Based on these findings, the method is considered precise and repeatable (Table 2).

Intermediate precision

An intermediate-precision (IP) study was conducted by repeating the MP parameters with different analysts, on different days, and using different column lots. The content and %RSD of the impurity were measured in both the sample and spiked solutions. The original sample solutions did not contain the impurity.

For the spiked sample solutions (n=6), the %RSD for the NDMA peak was 3.6%. The overall %RSD for all preparations (n=12) from both the method and intermediate-precision spiked samples at the specification level was less than 20.0%. Based on these findings, the method is considered robust. (Table 2).

Accuracy

An accuracy study was performed by spiking a known amount of NDMA into a sample over a range of concentrations, from the Limit of Quantification (LOQ) to 150% of the target level. The prepared solutions were made at four distinct concentrations: LOQ, 50%, 100%, and 150% of the target value. Each concentration was prepared in triplicate and injected once. The percentage recovery of the NDMA content was then calculated from these spiked samples. The method was considered accurate because the recovery results for all concentration levels fell within the acceptable range of 80% to 120% (Table 2).

Robustness

The robustness parameter was used to confirm the method's robustness to slight changes in the final method. By changing column flow rate plus (+) flow 0.9 mL/min, minus (-) flow 0.7mL/min and column oven temperature changes to plus (+) column oven temperature at 32°C and minus (-) column oven temperature at 28°C, the results were compared with standard and spike solution at specification levels of method precision (MP) for retention time (RT) and concentration of NDMA. The % difference in NDMA content between the results obtained in the method precision and robustness study was less than 5%, and the analyte retention time variation was ≤ 0.5 min (Table 2).

Solution stability

The stability studies were performed using a secondary intermediate stock solution of NDMA and spiked samples. The samples were stored at 100% concentration levels up to 48 hours at both ambient laboratory temperature (25 ± 5 °C) and under refrigerated conditions (2–8 °C). The percent recoveries of primary standard solutions of NDMA and spiked

Table 2. Method validation results summary.

Validation parameters	Typical acceptance criteria	Results
Method precision	RSD (%) for six preparations (n = 6) of spiked sample at specification level should be ≤ 15.0	3.2
Intermediate precision	RSD (%) for six preparations (n = 6) of spiked sample at specification level should be ≤ 15.0	3.6
	RSD (%) for preparations (n = 12) of MP and IP spiked sample at specification level should be ≤ 20.0	≤ 20.0
Accuracy	LOQ average recovery (n = 3) should be between 80 to 120%.	91.0
	50% average recovery (n = 3) should be between 80 to 120%.	92.2
	100% average recovery (n = 3) should be between 80 to 120%.	92.6
	150% average recovery (n = 3) should be between 80 to 120%.	95.8
Robustness	Plus (+) flow 0.9 mL/min: spiked sample concentration % difference and retention time	1.8% 1.8 min
	Minus (-) flow 0.7 mL/min: spiked sample concentration % difference and retention time	1.7% 2.2 min
	Plus (+) oven 32°C: spiked sample concentration % difference and retention time	2.8% 1.9 min
	Minus (-) oven 28°C: spiked sample concentration % difference and retention time	2.4% 2.1 min
Solution Stability	Standard and 100% spiked solution stored at ambient laboratory conditions (25 ± 5°C) and refrigerated conditions (2–8°C) were studied for 48 hours	Solutions are Stable for 48 hours

samples subjected to stability studies were calculated by comparing their results to freshly prepared primary standard solutions of NDMA (Table 2).

Discussion

An ultraperformance liquid chromatography with triple quadrupole mass spectrometry is a powerful analytical technique for highly specific and quantitative measurements of very low levels of analytes and impurities determination in pharmaceutical industry. An optimized UPLC–MS/MS method was developed to determine the NDMA content in Ibrutinib. Since molecular mass is more specific to each compound, no interference was observed at the analyte's retention time from other drug substances or blanks. An advantage of this method is the detection of NDMA at ppm to ppb levels, whereas the reported methods [17-20] were silent on the content and determination of NDMA in ibrutinib. The method offers several advantages over the reported available methods. The use of UPLC–MS/MS provides a more sensitive and reproducible approach. Further, the proposed method demonstrated high accuracy and precision during the validation study. Sensitivity was evaluated using the LOQ, set at 0.01 ppm. Based on the above, the data indicate that this method is compatible with and superior to those reported in the other published articles.

Conclusion

A sensitive, selective, and rapid UPLC-MS/MS method was developed for the identification and quantification of NDMA in Ibrutinib samples. This technique is highly sensitive, capable of detecting trace levels and identifying NDMA at parts-per-million (ppm) levels, thanks to advanced technology. To meet industrial and regulatory requirements, the method underwent validation in accordance with ICH, FDA, and U.S. Pharmacopeia (USP) guidelines. The validation confirmed the method is specific, linear, precise, accurate, rugged, and robust, demonstrating its reliability for obtaining consistent data in future experiments.

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Authors' contributions

Anuradha Bhimireddy and J V Shanmukha Kumar designed the research study and contributed research ideas. All authors reviewed the literature and collected the necessary data. All authors reviewed and approved the final manuscript.

Consent for publication

Not applicable.

Availability of data and material

The data are available from the author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Conflict of Interest

The authors declare that there is no conflict of interest, financial or otherwise. This article does not contain any studies with human participants, animal subjects, or plants performed by any of the authors.

References

1. Beard, J.C.; Swager, T.M. An Organic Chemist's Guide to N-Nitrosamines: Their Structure, Reactivity, and Role as Contaminants. *J. Org. Chem.* 2021, 86, 2037-2057. https://doi.org/10.1021/acs.joc.0c02774

2. Dobo, K.L.; Kenyon, M.O.; Dirat, O.; Engel, M.; Fleetwood, A.; Martin, M.; Mattano, S.; Musso, A.; McWilliams, J.C.; Papanikolaou, A.; Parris, P.; Whritenour, J.; Yu, S.; Kalgutkar, A.S. Practical and Science-Based Strategy for Establishing Acceptable Intakes for Drug Product N-Nitrosamine Impurities. *Chem. Res. Toxicol.* 2022, 35, 475-489. https://doi.org/10.1021/acs.chemrestox.1c00369

3. Thomas, R.; Tennant, R.E.; Oliveira, A.A.F.; Ponting D.J. What Makes a Potent Nitrosamine? Statistical Validation of Expert-Derived Structure-Activity Relationships. *Chem. Res. Toxicol.* 2022, 35, 1997-2013. https://doi.org/10.1021/acs.chemrestox.2c00199

4. Pu. C.; Zeng. T. Comparative Evaluation of Chemical and Photolytic Denitrosation Methods for Chemiluminescence Detection of Total N-Nitrosamines in Wastewater Samples. *Environ Sci Technol.* 2023, 57, 7526-7536. https://doi.org/10.1021/acs.est.2c09769

5. Xie, B.; Guo, D.; Mai, B.; Fan, J. Determination of Genotoxic Impurity N-Nitroso-N-methyl-4-aminobutyric Acid in Four Sartan Substances through Using Liquid Chromatography-Tandem Mass Spectrometry. *Molecules.* 2022, 27, 7498. https://doi.org/10.3390/molecules27217498

6. Kroes, R.; Renwick, A.G.; Cheeseman, M.; Kleiner, J.; Mangelsdorf, I.; Piersma, A.; Schilter, B.; Schlatter, J.; van Schothorst. F.; Vos, J.G.; Würtzen, G.; European branch of the International Life Sciences Institute. Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet. *Food Chem Toxicol*. 2004, 42, 65-83. https://doi.org/10.1016/j.fct.2003.08.006

7. Gerding, J.; Anhäuser, L.; Eickmann, U.;

Nienhaus, A. A simple approach to assess the cancer risk of occupational exposure to genotoxic drugs in healthcare settings. *J Occup Med Toxicol* 2022, 17, 8. https://doi.org/10.1186/s12995-022-00349-z

8. Wichitnithad, W.; Nantaphol, S.; Noppakhunsomboon, K.; Rojsitthisak, P. An update on the current status and prospects of nitrosation pathways and possible root causes of nitrosamine formation in various pharmaceuticals. *Saudi Pharm J.* 2023, 31, 295-311. https://doi.org/10.1016/j.jsps.2022.12.010

9. Nam, H.Y.; Nam, J.H.; Yoon, G.; Lee, J.Y.; Nam, Y.; Kang, H.J.; Cho, H.J.; Kim, J.; Hoe, H.S. Ibrutinib suppresses LPS-induced neuroinflammatory responses in BV2 microglial cells and wild-type mice. J Neuroinflammation. 2018, 15, 271. https://doi.org/10.1186/s12974-018-1308-0

10. Szklener, K.; Michalski, A.; Żak, K.; Piwoński, M.; Mańdziuk, S. Ibrutinib in the Treatment of Solid Tumors: Current State of Knowledge and Future Directions. *Cells*. 2022, 11, 1338. https://doi.org/10.3390/cells11081338.

11. Dong, R.; Yan, Y.; Zeng, X.; Lin, N.; Tan, B. Ibrutinib-Associated Cardiotoxicity: From the Pharmaceutical to the Clinical. *Drug Des Devel Ther.* 2022, 16, 3225-3239. https://doi.org/10.2147/DDDT.S377697

12. Liu, W.; Huang, J.; Yan, Z.; Lin, Y.; Huang, G.; Chen, X.; Wang, Z.; Spencer, P.S.; Liu, J. Association of N-nitrosodimethylamine exposure with cognitive impairment based on the clues of mice and humans. *Front Aging Neurosci*. 2023, 15, 1137164. https://doi.org/10.3389/fnagi.2023.1137164

13. Mansouri I, Botton J, Semenzato L, Haddy N, Zureik M. N-nitrosodimethylamine-Contaminated Valsartan and Risk of Cancer: A Nationwide Study of 1.4 Million Valsartan Users. *J Am Heart Assoc.* 2022 Dec 20;11(24):e8067. https://doi.org/10.1161/JAHA.122.026739

14. Chittireddy, H.N.P.R.; Kumar, J.V.S.; Bhimireddy, A.; Shaik, M.R.; Shaik, A.H.; Alwarthan, A.; Shaik, B. Development and Validation for Quantification of Cephapirin and Ceftiofur by Ultraperformance Liquid Chromatography with Triple Quadrupole Mass Spectrometry. *Molecules* 2022 Nov 16;27(22):7920. https://doi.org/10.3390/molecules27227920

15. Chittireddy, H.N.P.R.; Kumar, J.V.S.; Bhimireddy, A.; Shaik, M.R.; Khan, M.; Adil, S.F.; Khan, M.; Aldhuwayhi, F.N. Development and Validation for Quantification of 7-Nitroso Impurity in Sitagliptin by Ultraperformance Liquid Chromatography with Triple Quadrupole Mass Spectrometry. Molecules 2022, 27, 8581. https://doi.org/10.3390/molecules27238581

16. Chittireddy, H.N.P.R.; Kumar, J.V.S.; Bhimireddy, A.; Shaik, M.R.; Khan, M.; Khan, M.; Oh, T.H.; Shaik, B. Development and Validation of Analytical Method Using Gas Chromatography with Triple Quadrupole Mass Spectrometry for the Detection of Alkyl Halides as Potential Genotoxic Impurities in Posaconazole. *Separations* 2023, *10*, 295. https://doi.org/10.3390/separations10050295

17. Karvaly, G.B.; Vincze, I.; Balogh, A.; Köllő, Z.; Bödör, C.; Vásárhelyi, B. A High-Throughput Clinical Laboratory Methodology for the Therapeutic Monitoring of Ibrutinib and Dihydrodiol Ibrutinib. *Molecules* 2022, 27, 4766. https://doi.org/10.3390/molecules27154766

18. Fouad, M.; Helvenstein, M.; Blankert, B. *J. Anal. Methods in Chem.* 2015 (2015), 215128. https://doi.org/10.1155/2015/215128

19. Dunleavy, K.; Lai, C.E.; Roschewski, M.; Brudno, J.N.; Widemann, B.; Pittaluga, S.; Jaffe, E.S.; Lucas, A.N.; Stetler-Stevenson, M.; Yuan, C.M.; et.al., *Blood* 2015, 126, 472. https://doi.org/10.1182/blood.V126.23.472.472

20. Grommes, C.; Pastore, A.; Gavrilovic, I.; Kaley, T.; Nolan, C.; Omuro, A.M.; Wolfe, J.; Pentsova, E.; Hatzoglou, V.; Mellinghoff, I.; DeAngelis, L.; *Blood* 128, 783 (2016). https://doi.org/10.1182/blood.V128.22.783.783

21. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use ich harmonised tripartite guideline validation of analytical procedures: text and methodology Q2(R1). https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf

22. Analytical Procedures and Methods Validation for Drugs and Biologics Guidance for Industry, https://www.fda.gov/files/drugs/published/Analytical-Procedures-and-Methods-Validation-for-Drugs-and-Biologics.pdf

23. USP <1225> Valıdatıon of compendıal methods, http://www.uspbpep.com/usp29/v29240/usp29nf24s0_c1225.html